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Changes in Biochemical and Physiological Indices in Animals Produced by the Combined Effect of Benz [a] pyrene and Phenol

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Early energy changes in lungs, liver, and kidneys during the introduction of benz a pyrene and phenol (as the possible carcinogen activator) were studied. It was observed that 10 days after a single instance introduction of 5 mg benz a pyrene per 0.9% NaCl (60 rats), oxidative phosphorylation in the lungs and livers is disturbed in the test rats, accompanied by a reduction of adenine nucleotides in these tissues. It is assumed that at this stage, the detoxication of benz a pyrene is intensified by free oxidation systems and by the respiratory chain of mitochondria.

A chronic 3-month exposure to benz a pyrene and phenol (150 rats, each 5 mg of benz a pyrene per month intratracheally and 0.4 mg/m^3 of phenol round-the-clock) results in greater disturbances of the energy exchange in the lungs, liver and kidneys.

Benz a pyrene and phenol, individually and in combination, inhibit oxidative phosphorylation in the lungs. This significantly decreases the content of adenine nucleotides in this tissue. Activation of anaerobic glycolysis (twofold) and of aerobic glycolysis (eightfold) does not make up for the energy insufficiency in the tissue. The effect of benz a pyrene and phenol in the liver also results in suppressing oxidative phosphorylation and in the activation of glycolysis (anaerobic 2.5 times, the aerobic 3.7 times). Changes in the bioenergy of the kidneys are not as great.

Phenol in its combined effect with benz[a]pyrene intensifies the effect of the latter, as shown primarily to the greater activation of anaerobic and aerobic glycolysis in the lungs and livers of test rats.

The observed disturbances as concern the weight dynamics of the animals (weight loss in test rats), vitamin metabolism (their decrease in the organs and in urine) and hemopoiesis of red blood cells (erythropenia) attest to the toxic effect of benz a pyrene phenol on the organism, which is greater in the case of the combined action of the studied agents. No changes were discerned in the morphology of white blood cells.

The intense pollution of the air of modern industrial cities by carcinogens and to a greater extent by accompanying toxic substances constitutes a problem associated with the study of the role of atmospheric pollution in lung carcinogenesis. The solution of this problem will make it possible to establish a basis for evaluating the danger of possible provoking atmospheric factors in the etiology of lung cancer and to approach the evaluation of the long-term after effects of the action of atmospheric pollutants on the population.

Previous studies conducted by the authors in 1965–67 (*1*) show that the atmospheric pollutant, sulfur dioxide, can stimulate the tumor process in the lungs during chronic inhalation. Our data are in agreement with the research results of Kuschner

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Table 1.

Group	No. of rats	Exposure	Single dose of benzpyrene mg	Total dose of benzpyrene, mg	Average concentration of phenol, mg/m³	Exposure time, months: Benzpyrene	Exposure time, months: Phenol	Overall exposure time, months
I	50	Benzpyrene + phenol	5	30	0.4	6	6	6
II	50	Benzpyrene followed by *phenol*	5	15	0.4	3	3	6
III	50	Benzpyrene	5	30	—	6	—	6
IV	40	Phenol	—	—	0.4	—	6	6
V	40	BK-8	Once per month intratracheally, 0.2 ml BK-8 and 0.2 mg India ink					
VI	30	Control	—	—	—	—	—	—

and Laskin (*2, 3*), whose data showed that a greater number of lung tumors are evoked by the long-term inhalation of a sulfur dioxide and benzpyrene mixture than by the inhalation of a single carcinogen.

At present, we are studying the cocarcinogenic or activating properties of another atmospheric pollutant, phenol, in the blastomogenesis of the lungs.

With this in mind, a test was set up using 260 random-bred rats of both sexes, 5–6 months old. The animals constituted six groups with two test groups and four control groups. The design of the experiment is given in Table 1.

In conducting the experimental work, two methods of exposure were used: round-the-clock inhalation exposure to phenol vapor, and intratracheal instillation of a 5 mg benzpyrene dose monthly. The methods of Shabad (*4*) (1962) and Pylev (*5*) were used to introduce the carcinogen intratracheally. The benzpyrene was introduced into the lungs in the form of a suspension in BK-8 plasma expander. Finely ground, powdered India ink was added to the suspension for marking purposes and to create a carcinogen deposit in the lungs. The animals were exposed to phenol at a concentration of 0.4 mg/m³ in 100-liter chambers, where the animals remained continuously throughout the experiment, except when blood samples were taken from the animals and the animals were otherwise manipulated (intratracheal introduction, weighing, etc.).

The following indices of the animals were determined during the course of the experimental period: weight of the animals, erythrocyte and leucocyte count and the differential white count, induction time for subcutaneous tumors, vitamin content in the organs and in the urine, extent of aerobic and anerobic glycolysis and oxidative phosphorylation in the tissues, and pathomorphology.

This report contains the results of biochemical and physiological studies carried out on animals which had been exposed to the combined action of a carcinogen and phenol.

Of greatest interest is research dealing with the study of the energy changes in the organs of animals. Previous studies showed that sulfur dioxide has a significant effect on energy change. The combination of benzpyrene and sulfur dioxide introduced into the lungs of rats in our experiments resulted in a marked intensification of anaerobic and aerobic glycolysis in the lung and liver homogenates of these animals, in a reduction in the amount of adenine nucleotides in these tissues, in the appearance of leukemia and tumors in various sites in more instances than was the case with a single carcinogen (*1, 6–8*).

In conjunction with this, the aim of this portion of work is the study of the early shifts in energy changes evoked by the introduction into the organism of the carcinogenic hydrocarbon, 3,4-benzpyrene (BaP). This hydrocarbon is most often encountered in the air of cities. The objective of this study is also the evaluation of one of the most widespread atmospheric pollutants, phenol, and its possible role in intensifying the effect of benzpyrene.

The investigation was divided into two stages: a study of the action of 3,4-benzpyrene on the oxidative phosphorylation and glycolysis of the mitochondria and homogenates of some tissues in rats after a single introduction of a carcinogen into the lungs of the test animals and a study of energy changes in the tissues of test animals after long-term exposure to benzpyrene, phenol, or a combination of these substances. In formulating this work and in evaluating its results, the author's work was based on the concept evolved by Warburg (*9, 10*) that disturbance of the energy changes in a cell serves as the basis of the neoplastic process of tissue.

The work was carried out using 4-8 month old, white, male rats. Short-term exposure was used in order to study the effect of 3,4-benzpyrene on energy changes. The rats were given a single 5-mg dose of benzpyrene intratracheally in the form of a 0.9% NaCl suspension. The energy change in the animal tissue was studied 10 days after exposure. The series utilized 60 rats.

Concurrently, a long-term exposure of the animals was carried out according to the scheme described above, with the one exception that the animals were exposed to benzpyrene and phenol during the course of 3 months.

After completion of the exposure period, a month after the last introduction of benzpyrene, the animals were used in tests to study energy changes. This stage utilized 150 rats.

The total amount of benzpyrene received by the rats during the long-term exposure constituted 15 mg or 60 μ mole per animal (6×10^{-5} mole).

Energy changes were determined by using three parameters. The content of the components of glycolysis of adenylic system and inorganic phosphate in the tissues of the lungs, liver, and kidneys of control and test animals were determined after slowly fixing in liquid nitrogen after decapitation and tissue pulverization; Anaerobic and aerobic glycolysis in the homogenates of these tissues was studied. Also oxidative phosphorylation in the mitochondria of the liver and kidneys and in the homogenates of the lungs from the control and test animals was followed.

The medium used to obtain homogenate for studying glycolysis contained Tris buffer, $0.05M$, pH 7.3; HCl, $0.15M$; nicotinamide, $5 \times 10^{-3}M$; $MgCl_2$, $6 \times 10^{-3}M$. The homogenization took 3 minutes in a homogenizer with a Teflon pestle. The incubation medium contained Tris buffer, $0.05M$, KCl, $0.15M$, $MgCl_2$, $6 \times 10^{-3}M$; K_2HPO_4, $0.03M$, ATP (adenosine triphosphate), $2 \times 10^{-3}M$; NAD (nicotinamide adenine dinucleotide), $2.5 \times 10^{-4}M$, and cysteine $2 \times 10^{-3}M$. The substrate consisted of glycogen in a final concentration of $0.3M$ for the liver and kidneys, and fructose-1,6-diphosphate (FDP), $0.01M$, for the lungs. The study of glycolysis in the homogenate of the lungs was conducted on a shortened glycolytic chain, since it was pointed out earlier that the phosphorylase and hexokinase in the lungs are not very active. Incubation was for 30 min at 30°C with a gas phase of nitrogen and air. The following were determined: glycogen consumption, increase of FDP and lactate in the samples with the homogenates of the liver and kidneys and the increase in consumption of FDP and lactate in the samples with the homogenates of the lungs.

Oxidative phosphorylation was studied by Warburg's gasometric method (*9, 10*) in the mitochondria of the liver and kidneys and in the homogenates of the lungs. The medium for isolating the mitochondria contained Tris buffer, $0.05M$, pH = 7.4; versene, $0.001M$; and sucrose, $0.25M$. The incubation mixture for the Warburg was: Tris buffer, $0.05M$, versene, $0.001M$; phosphate, $0.03M$; $MgCl_2$, $0.01M$; KCl, $0.15M$, fluoride, $0.01M$, succinate, $0.04M$; glucose, $0.1M$; ATP, $0.001M$, and hexokinase, 0.7 mg/sample. In addition, cytochrome c, $8.4 \times 10^{-4}M$, was introduced for the lungs. The terminal pH was 7.4. The incubation lasted 30 min at 30°C with air as the gas phase.

The determination of the content of the components of glycolysis and of the adenylic systems in nitrogen fixed tissues included the determination of glycogen, FDP (fructose diphosphate), lactic acid, inorganic phosphate, and the adenylnucleotides ATP, ADP, and AMP.

The glycogen was determined according to the Krisman method with modification by the authors. This modification involved the use of FDP with resorcinol, lactic acid according to Barker and Sommerson, inorganic phosphate according to Fisk-Subbarrow, ATP, ADP, and AMP by the chromatographic method according to Carter with subsequent quantitative determination by using ribose. The albumin was determined by the microbiuret method.

All the obtained results were converted to grams of raw tissue weight (glycolysis and determination in the tissues) or to milligrams of albumin (oxidation phosphorylation). The results were statistically processed according to the method of Van der Varden, Student and mutually coupled variants.

Ten days after the introduction of 5 mg benzpyrene (BP) into the lungs of experimental rats in a single dose, a disturbance takes place in the oxidative phosphorylation in the lungs and liver. This results in the reduction of the quantity of adenine nucleotides in these tissues. At the same time, oxidative phosphorylation in the kidneys is reduced to a lesser extent (Tables 2–4). At this stage, intensification most likely takes place in the detoxication of benzpyrene by the systems of free oxidation and by the respiratory chain of the mitochondria. The enzymes of glycolysis appear to be in an activated state in comparison to the control group (by 20%), (Tables 2–4). This is ascertained from a study of glycolysis in the homogenates of the tissues during the introduction of NAD into the incubation medium. The observed changes most likely correspond to that stage when the regulator reaction of the organism still is operative.

The long-term exposure of animals to benzpyrene, phenol, and their combination results in a severe disturbance of the energy changes in all of the three tissues studied.

Benzpyrene and phenol alone or in combination inhibit oxidative phosphorylation in the mitochondria of the lungs. This results in the significant reduction of the amount of adenine nucleotides in this tissue. The activation of glycolysis, both anaerobic (2.0 times) and aerobic (8 times) cannot compensate for the lack of energy in the tissue (Tables 2–4).

The nature of the action of benzpyrene and phenol is somewhat different in the liver than in the lungs, despite the fact that oxidative

Table 2. Glycolysis in the homogenates of tissues after single introduction of benzpyrene and/or phenol.

		Parameters reflecting glycolysis, % of control values					
		After 10 days			After 3 months		
	Exposure	Lungs	Liver	Kidneys	Lungs	Liver	Kidneys
Anaerobic							
Glycogen (consumption)	BP	—	89.5[a]	122.0	—	102.0	101.0
	Phenol	—	—	—	—	95.0	153.0[a]
	BP + phenol	—	—	—	—	108.0	144.0[a]
FDP (fructose diphosphate)	BP	96.0	88.0	69.2[a]	160.0[a]	154.0[a]	40.5[a]
	Phenol	—	—	—	256.0[a]	145.0[a]	61.5[a]
	BP + phenol	—	—	—	192.0[a]	210.0[a]	57.2[a]
Lactate	BP	119.0	120.0	123.0	107.0	210.0[a]	60.5
	Phenol	—	—	—	81.0	222.0[a]	175.0
	BP + phenol	—	—	—	92.6	262.0[a]	102.0
Aerobic							
Glycogen (consumption)	BP	—	82.9	—	—	118.0	80.5
	Phenol	—	—	—	—	41.6[a]	190.0
	BP + phenol	—	—	—	—	125.0[a]	155.0
FDP	BP	103.0	119.0[a]	210[a]	90.5	146.0[a]	273.0[a]
	Phenol	—	—	—	133.0	135.0	50.8
	BP + phenol	—	—	—	860.0[a]	138.0[a]	71.0
Lactate	BP	93.2	129.0[a]	129.0	117.0[a]	316.0[a]	154.0[a]
	Phenol	—	—	—	127.0[a]	292.0[a]	45.0
	BP + phenol	—	—	—	116.0[a]	376.0[a]	76.3

[a]Statistically significant changes.

Table 3. Oxidation phosphorylation in lung homogenate and the mitochondria of liver and kidneys after single introduction of benzpyrene and/or phenol.

		Parameters of oxidative phosphorylation, % of controls					
		After 10 days			After 3 months		
	Exposure	Lungs	Liver	Kidneys	Lungs	Liver	Kidneys
Absorption of phosphate							
ΔP	BP	71.5[a]	88.0[a]	78.3	44.3[a]	37.0[a]	27.0[a]
	Phenol	—	—	—	152.0[a]	130.0[a]	30.0[a]
	BP + phenol	—	—	—	55.5[a]	28.6[a]	61.0
Absorption of oxygen							
ΔP	BP	74.0	162.0[a]	88.5[a]	79.0	53.5[a]	45.0[a]
	Phenol	—	—	—	47.5[a]	41.7[a]	53.5[a]
	BP + phenol	—	—	—	76.5	53.3[a]	62.5[a]
Coefficient of coupling							
P/O	BP	96.0	53.0	88.5	57.6[a]	70.8[a]	63.0[a]
	Phenol	—	—	—	105.0	315.0[a]	55.0[a]
	BP + phenol	—	—	—	71.0	53.5[a]	98.0

[a]Statistically significant changes.

Table 4. Content of adenine nucleotides in the lungs, liver, and kidneys after single introduction of benzpyrene and/or phenol.

Nucleotide	Exposure	Nucleotide content, % of controls					
		After 10 days			After 3 months		
		Lungs	Liver	Kidneys	Lungs	Liver	Kidneys
ATP							
	BP	66.5[a]	77.2[a]	65.3	74.0[a]	50.7[a]	50.0[a]
	Phenol	—	—	—	114.0[a]	75.8[a]	100.0
	BP + phenol	—	—	—	78.5[a]	68.7[a]	70.5
ADP							
	BP	55.6[a]	90.0	124.0	63.0	64.7[a]	76.2[a]
	Phenol	—	—	—	100.0	106.0	114.0
	BP + phenol	—	—	—	100.0	102.0	78.5
AMP							
	BP	81.5[a]	91.0	108.0	45.5[a]	59.0[a]	98.0
	Phenol	—	—	—	100.0	83.0[a]	135.0[a]
	BP + phenol	—	—	—	77.3	77.3[a]	124.0[a]
Sum of adenine nucleotides							
	BP	81.7[a]	85.0[a]	94.0	65.8[a]	56.8[a]	75.0[a]
	Phenol	—	—	—	108.0	85.8	110.0
	BP + phenol	—	—	—	82.5[a]	80.0[a]	91.8

[a]Statistically significant changes.

phosphorylation and glycolysis activation are also suppressed (2.5 times in the case of the anaerobic process and 3.7 times in the case of the aerobic process).

Changes in the bioenergetics were evident to a lesser extent in the kidneys. Benzpyrene and phenol inhibit oxidative phosphorylation as a whole, along with respiration and phosphorylation. This results in a decrease to some extent, of the amount of adenine nucleotides in this tissue.

These changes in oxidative phosphorylation in the mitochondria of the kidneys did not evoke any significant intensification of the glycolysis in the homogenate of the kidneys (except for the benzpyrene group where the aerobic glycolysis is activated 1.5 times).

The results of the studies of the physiological indices, particularly the weight dynamics of the animals, indicate that the least weight increase (in per cent with respect to the initial weight) in comparison to the control group (group VI of Table 1) was observed in the animals in groups I, II, and III, in both males and females. In addition, the combined action of benzpyrene and phenol on the organism is greater than is that of either of them individually. The changes in weight dynamics were greater in the females than the males.

Studies of the morphological composition of peripheral blood, showed that erythropenia in the animals which had been subjected to the effect of phenol and benzpyrene (groups II and IV); this was particularly evident in the females.

In the given case the specific action of phenol took place. Statistically significant erythropenia was observed in the case of the female rats of group III which had received only benzpyrene.

Analysis of the hemograms of the white blood both of the experimental and of the control groups indicated that there was a significant variation in the total number of leucocytes, lymphocytes, and segmented neutrophils. The statistical treatment of the hemograms did not show a difference between the test and control groups.

Research on the vitamin (C, B_1, B_2, PP and their derivatives) content in organs and urine indicates that more severe disturbances in vitamin metabolism are observed in the case of the combined action of a carcinogen and phenol than is the case with the action of these substances individually.

Results of the biochemical and physiological studies may be summarized as follows.

Benzpyrene evokes a disturbance of the bioenergetics in the tissues of lungs, liver, and kidneys immediately after introduction. These disturbances are intensified with time and with increased benzpyrene dose.

The intensifying effect of phenol was determined primarily by its greater activation of the anaerobic glycolysis and particularly of aerobic glycolysis in the combined exposure group in the homogenates of the lungs and liver.

The indicated disturbances with respect to the weight dynamics of the animals, vitamin metabolism, and hemopoiesis of red blood verify the toxic effect of a carcinogen and phenol on an organism. These disturbances are particularly marked in the case of the combined action of the studied agents. No changes in the morphology of white blood cells were observed.

REFERENCES

1. Skvortsova, N. N., et al. The combined effect of small concentrations of sulfur dioxide and benzpyrene on animal organisms in chronic experiments. In: Materialy Konferentsii po Itogam Nauchnykh Issledovanii za 1967G, Instituta Obshehei i Kommunal'noi Gigieny im. A. N. Sysina Akad. Nauk Med. S.S.S.R. (Proceedings of the Conference on the Research Results for 1967), Institute of General and Communal Hygiene imeni A. N. Sysin of the U.S.S.R. Academy of Medical Sciences), Moscow, 1968, p. 9.
2. Kuschner, M. The causes of lung cancer (J. Burns Amberson Lecture). Amer. Rev. Resp. Dis. 98: 573 (1968).
3. Kuschner, M. and Laskin, S. Experimental models in environmental carcinogenesis. Amer. J. Path. 64: 183 (1971).
4. Shabad, L. M. Experimental lung cancer. Arkhiv Patol. 1962: No. 6, p. 3.
5. Pylev, L. N. Experimental Production of lung cancer in rats with 3, 4-benzpyrene. Vestn. Akad Med. Nauk S.S.S.R. II: 41. (1964).
6. Skvortsova, N. N. and Osintseva, V. P. The possible role played by sulfur dioxide in the etiology of pulmonary, chemical blastomogenesis. In: Materialy Respubliknskii Nauchno-Prakticheskii Konferentsii po Problemye Gigieny v Usloviyakh Uzbekistana. (Proceedings of the Republic Scientific-Practical Conference on the Problem of Hygiene Under Conditions in Uzbekistan). Tashkent, 1970, p. 1962.
7. Skvortsova, N. N., et al. Problem associated with blastomogenesis in the lungs of rats under the combined effect of sulfur dioxide and a carcinogen. In: Nekotorye Itogi Izucheniya Zagrayazneniya Vneshnyei Sredy Kantserogennymi Veshchestvami. (Some Results From Studying the Pollution of the Environment With Carcinogens). Proceedings of an Expanded Plenary Session of the Committee on Carcinogens and Preventive Practice of the Main Sanitary and Epidemiological Administration of the U.S.S.R. Ministry of Health, L. M. Shabad, Ed., Moscow, 1972, p. 20.
8. Vysochina, I. V., Konstantinova, I. N., and Skvortsova, N. N. Effect of sulfur dioxide on glycogenolysis in the lungs and liver. Gigiena i Sanit. 41: No. 4, 3 (1974).
9. Warburg, O. Uber die Entstehung der Krebszellen. Onkologia 9: 75 (1956).
10. Warburg, O. Weitentwikulung der Zellphysiologisehen Methoden, angwandt auf Krebs. Photosyntese und Wiekungsweise der Röntgenstrahlen, Stuttgart, 1962.